HLA Antigens in Turkish Race With Rheumatic Heart Disease

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Background. Rheumatic valvular disease has been reported to be associated with HLA antigens. To determine whether genetic factors could be involved in the pathogenesis of rheumatic heart disease (RHD), we analyzed the distribution of HLA-A, HLA-B, and HLA-DR antigens in Turkish patients with chronic rheumatic heart disease.

Methods and Results. The association of class I and class II HLA antigens was examined in 107 ethnic Turkish patients with chronic RHD. The diagnosis was supported by echocardiography, cardiac catheterization, angioventriculography, and histological findings in patients who underwent valve replacement. Two hundred three control subjects, also of Turkish origin, were chosen. The phenotypes B16, DR3, and DR7 were encountered in a significantly higher frequency in patients with RHD compared with the control population (corrected p < 0.005, p < 0.00005, and p < 0.0005, respectively). There also was a decrease in the antigen frequency of DR5 in patients compared with controls (corrected p < 0.005).

Conclusions. The results are consistent with the hypothesis that susceptibility to RHD is genetically linked, and this in turn may be associated mainly with HLA class II antigens and weakly with class I antigens, with DR3, DR7, and B16 influencing susceptibility and DR5 conferring protection. (Circulation 1993;87:1974–1978)

KEY WORDS • genetics • rheumatic valvular disease • Turkish race • antigens

The HLA antigens, which are encoded by closely arranged genes on the short arm of chromosome 6, influence predisposition to several diseases.1 Rheumatic valvular heart disease (RHD) has been postulated to have autoimmune features,2,3 and many investigators have postulated an important role of inherited susceptibility in the causation of RHD. Their observations are based on epidemiological surveys indicating familial occurrence of the disease4 and the reported association with HLA-A, HLA-B, HLA-C, and HLA-D or HLA-DR loci. Although a simple autosomal recessive pattern of inheritance has been suggested,5 attempts to identify genetic markers of susceptibility in RHD have been only partially successful. Furthermore, the reported data indicate that the HLA associations appear to be related to racial and ethnic extraction. Thus, we thought it worthwhile to study a group of Turkish patients with RHD for possible HLA associations.

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Methods

Study Population

The study was conducted on 107 patients of either sex with established RHD who were admitted to the cardiology and cardiovascular surgery wards of the Koşuyolu Heart and Research Institute, Istanbul. There were 64 female and 43 male patients between 17 and 58 years old (mean age, 34.2±10.45 years; [SD]). All patients were ethnic Turks. The diagnosis was chiefly made by observing clinical findings compatible with RHD in physical examination and as suggested by echocardiography; the majority of patients also had cardiac catheterization. The histological examination of the valves obtained during cardiac surgery in 76 of the patients also was contributory to the diagnosis, showing scarring consistent with RHD. Fifty-five patients had mitral valve disease; 50 had pure stenosis. Three had aortic valve disease, 41 had involvement of both mitral and aortic valves, and eight had triple-valve involvement (mitral, aortic, and tricuspid).

The control group consisted of 203 normal organ donors of Turkish ethnicity who lived in the same geographic location. Tissue typing for HLA antigens on control subjects and patients was performed in the same laboratory.

HLA Typing

HLA typing for class I (HLA-A, HLA-B, and HLA-C) and class II (HLA-DR) antigens was performed by the standard two-stage Terasaki microlymphocytotoxicity technique.6 These antigens were defined by 110 antisera (94 for class I and 16 for class II). The antisera used in
both patients and controls came from the same sources (most from Eurotransplant and some from Behring Berke and Biotest) but were not necessarily of the same batch.

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#### Statistical Analysis

Differences between in patients and control groups in frequencies of various HLA antigens were calculated using the standard $\chi^2$ method with Yates' correction. $p$ values were corrected ($p_c$) by multiplying them by 41 and 6, i.e., the total number of HLA class I and class II antigens studied, as is done by some researchers.\(^7\)-\(^10\) Relative risk was calculated according to the method of Svejgaard et al.\(^11\)

### Results

The prevalence rates of various class I and II HLA alleles found in the controls used in the present study and in three previously reported healthy control groups from our country\(^12\)-\(^14\) were similar. Table 1 depicts the frequency of the various class I alleles among the patients and compares them with those found in the control group. It is seen that B16 was significantly more prevalent among the patients even after the $p$ correction.

### Table 2. Frequency of HLA-DR Antigens Among Patients

<table>
<thead>
<tr>
<th>HLA</th>
<th>Control subjects (n=203)</th>
<th>Patients (n=107)</th>
<th>$\chi^2$</th>
<th>Relative risk</th>
<th>$p$ ($p_c$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR1</td>
<td>28</td>
<td>14</td>
<td>13</td>
<td>0.05</td>
<td>0.86</td>
</tr>
<tr>
<td>DR2</td>
<td>45</td>
<td>22</td>
<td>11</td>
<td>5.91</td>
<td>0.40</td>
</tr>
<tr>
<td>DR3</td>
<td>47</td>
<td>23</td>
<td>52</td>
<td>19.72</td>
<td>3.14</td>
</tr>
<tr>
<td>DR4</td>
<td>55</td>
<td>27</td>
<td>39</td>
<td>2.47</td>
<td>1.54</td>
</tr>
<tr>
<td>DR5</td>
<td>112</td>
<td>55</td>
<td>34</td>
<td>14.45</td>
<td>0.38</td>
</tr>
<tr>
<td>DR7</td>
<td>67</td>
<td>33</td>
<td>61</td>
<td>15.68</td>
<td>2.69</td>
</tr>
</tbody>
</table>
TABLE 3. HLA Studies in Patients With Acute Rheumatic Fever or Rheumatic Heart Disease

<table>
<thead>
<tr>
<th>Authors</th>
<th>Race of patients</th>
<th>No. of patients</th>
<th>Disease</th>
<th>No. of HLA antigens tested</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Falk et al20</td>
<td>Caucasian</td>
<td>76</td>
<td>ARF and/or RHD†</td>
<td>17</td>
<td>↓ A3</td>
</tr>
<tr>
<td>Caughey et al21</td>
<td>Caucasian</td>
<td>(eight non-Caucasian)</td>
<td>50</td>
<td>ARF±RHD†</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Maori</td>
<td>50</td>
<td>ARF±RHD†</td>
<td>32</td>
<td>No association</td>
</tr>
<tr>
<td>Leirisalo et al22</td>
<td>Caucasian</td>
<td>109</td>
<td>ARF (38% had carditis)</td>
<td>24</td>
<td>↑ Bw35</td>
</tr>
<tr>
<td>Joysey et al23</td>
<td>Caucasian</td>
<td>94</td>
<td>RHD</td>
<td>21</td>
<td>↑ Bw15 when compared with one group of controls but not when compared with two other control groups</td>
</tr>
<tr>
<td>Ward et al24</td>
<td>Caucasian</td>
<td>58</td>
<td>Acquired valvular disease:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No history of ARF</td>
<td>27</td>
<td>↑ Aw30/31, ↑ A29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>With history of ARF</td>
<td></td>
<td>No association</td>
</tr>
<tr>
<td>Murray et al25</td>
<td>Caucasian (Hispanic)</td>
<td>49</td>
<td>ARF (18% had carditis)</td>
<td>32</td>
<td>No association</td>
</tr>
<tr>
<td>Haffejee et al26</td>
<td>Black</td>
<td>53</td>
<td>RHD</td>
<td>42</td>
<td>↑ A25, ↑ A10, ↑ Bw41</td>
</tr>
<tr>
<td>Naito et al27</td>
<td>Japanese</td>
<td>127</td>
<td>RHD</td>
<td>45</td>
<td>↑ B7, ↓ Bw16</td>
</tr>
<tr>
<td></td>
<td>(111 MS)</td>
<td></td>
<td></td>
<td></td>
<td>↑ B7, ↓ Aw19 in MS</td>
</tr>
<tr>
<td>Jhinghan et al28</td>
<td>Hindu</td>
<td>134</td>
<td>RHD (78 cases with past history of ARF)</td>
<td>37</td>
<td>↑ Aw33, ↑ DR3, ↓ DR2</td>
</tr>
<tr>
<td>Rajapakse et al10</td>
<td>Saudi</td>
<td>40</td>
<td>25 RHD</td>
<td>45</td>
<td>↑ DR4§</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15 ARF (without carditis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ayoub et al29</td>
<td>Caucasian</td>
<td>24</td>
<td>ARF (86% RHD)</td>
<td>9</td>
<td>↑ DR4, ↓ DR6</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anastasiosu et al30</td>
<td>Caucasian</td>
<td>33</td>
<td>RHD</td>
<td>9</td>
<td>↑ DR4, ↓ DR6</td>
</tr>
<tr>
<td>Maharaj et al19</td>
<td>Black</td>
<td>120*</td>
<td>RHD</td>
<td>45</td>
<td>↑ DR1, ↑ DRw6§</td>
</tr>
<tr>
<td>Taneja et al21</td>
<td>Hindu</td>
<td>54</td>
<td>RHD</td>
<td>40</td>
<td>↓ DR2, ↑ DR3, ↑ DQw2, ↓ DR5</td>
</tr>
<tr>
<td>Guilherme et al32</td>
<td>Brazilian</td>
<td>40</td>
<td>ARF (90% had carditis)</td>
<td>57</td>
<td>↑ DR7, ↑ DRw53</td>
</tr>
</tbody>
</table>

RHD, rheumatic heart disease; ARF, acute rheumatic fever; MS, mitral stenosis.

*HLA-DR and HLA-DQ typing in 103 and 97 of these patients, respectively.
†Percentage with carditis not mentioned.
‡Corrected p significant.
§Only HLA-DR types.
¶Marginally increased.

(p < 0.05; 15% versus 5%). The apparent positive significant association with A11 and the apparent negative significant associations with A2 and Bw21 were not sustained after the p corrections.

Among the class II alleles studied (Table 2), there were significantly important positive associations with HLA-DR3 and HLA-DR7, whereas a negative association was observed with HLA-DR5. The apparent negative association with HLA-DR2 was not sustained after the p correction.

**Discussion**

The historical verification of acute rheumatic fever (i.e., the fulfillment of Jones' criteria) among our patients would, by definition, be retrospective and not very reliable. However, we believe our patients had RHD in that vast majority (104 of 107) had either pure mitral stenosis or bivalvular or multivalvular disease, lesions most often associated with RHD, especially in a developing country like Turkey. The histological findings in the valves obtained during surgery were also consistent with RHD in that they showed scarring consistent with RHD and they did not show pathology (i.e., hematoxylin bodies, myxomatous lesions) associated with other conditions that cause valvular disease. Aschoff's bodies, the hallmark of rheumatic valve pathology, were not seen among our patients. They are most often found in atrial appendages and rarely in papillary muscles. These structures obviously were not available to us for histological examination.

Although an abnormal immune response following streptococcal infection has been proposed as a potential cause of RHD, the exact mechanism is not clear. Although a streptococcal etiology of the disease has been proven by several lines of argument, the low conversion rates of streptococcal pharyngitis into rheumatic fever make the study of host factors, especially genetic susceptibility, relevant. Numerous investigators have suggested the involvement of HLA class I and class II antigens in causing susceptibility to RHD. The pres-
ent study, to our knowledge, is the largest attempt to look at this as well as studying class II alleles. The HLA alleles implicated in the association with susceptibility to rheumatic fever are somewhat different (Table 3).6,10,20-31 These different results could be due to one or more of the following. First, regarding variation in number and selection of patients, the difference might be due to the difference in the number of patients studied. The weaker the association is between a disease and HLA, the larger number of patients that may be needed to ensure significance. Furthermore, some grouped acute rheumatic fever (with or without carditis) and RHD together. We included only patients with well-documented RHD, so it is conceivable that the HLA association with ARF might be different than RHD.

Second, regarding lack of racial homogeneity, the frequencies of HLA antigens vary considerably among races. These antigens show a characteristic distribution in a given racial group. For example, HLA-DR5 is relatively high in our population (55%). It drops to 25% in African-Americans 15% in Saudi Arabs, 4% in Japanese, and 3% in Native Americans.

Third, regarding the use of too few specific antisera in identifying the HLA antigens as well as the presence of cross-reacting antibodies, we used 110 well-defined antisera in the present study. HLA-B16 antigen was found to the overrepresented (15% of patients versus 5% of control subjects), and Bw21 was underrepresented (10% versus 20%) in our series. The association of B16 differs from the report of Naito et al.27 who found it to be underrepresented. Underrepresentation of Bw21 is unusual in that it had not been previously described. The weak underrepresentation of A2 (37% versus 51%) is compatible with the study of Ayoub et al.,29 although their patients were black. Reduced frequency of DR2 (10% versus 22%) in patients with RHD is in conflict with the study of Ayoub et al.,29 whereas it is compatible with the findings of Zhinghan et al.28 and Taneja et al.,31 both of whom are from India. Interestingly, our findings of a high HLA-DR3 frequency in patients with (49% versus 23%) RHD is in line with the findings from India.

The distribution of the HLA alleles in our control group was similar to that encountered in three previous studies. However, it should be pointed out that the rather high prevalence of HLA-DR5 (55%) among the control subjects in the present study was not very pronounced, especially in DR typing, in the other smaller groups previously reported from Turkey. This, we admit, makes the negative HLA-DR5 association brought up in this study somewhat debatable. On the other hand, other investigators like Ayoub et al.29 (black patients) and Taneja et al.31 (Hindu patients) reported a similar negative association with HLA-DR5.

Finally, Guilherme et al.32 (Brazil) reported a significant increase in HLA-DR7, similar to that found in our population (5% versus 33%). Although one cannot categorically state that there is a racial similarity among the four populations, these findings suggest that the positive DR3 and DR7 and negative DR2 and DR5 associations with RHD may transcend racial barriers. Our study lacks HLA-DQ typing. More research must be done in this area, especially in view of the conflicting reports in the literature.6,31

The finding of significant positive and negative associations between RHD and HLA antigens indicates that susceptibility to RHD in Turkish patients is mainly class II and weakly class I mediated, with B16, DR3, and DR7 influencing susceptibility and DR5 conferring protection. Some investigators suggested the possible existence of susceptible and resistant human phenotypes in RHD.20-31 The present study provides further evidence in support of this hypothesis.

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